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Kristina A. Thayer, PhD
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Dear Dr. Thayer:

The Soy Nutrition Institute (SNI) welcomes the opportunity to comment on the *Draft CERHR Expert Panel Report on Soy Formula* that was made available to the public on October 19, 2009. Our comments are rather brief because the SNI believes that although a considerable amount of research published since soy infant formula was evaluated by the CERHR in 2006 was identified by the panel, evaluation of this new information does not warrant a different conclusion about soy infant formula than was reached by the expert panel in 2006. Namely, that "There are insufficient human or experimental animal data available to permit a determination of the developmental or reproductive toxicity of soy infant formula."

The SNI is a science-based organization dedicated to promoting an accurate understanding of the impact of consuming soyfoods, soy oil and other soybean components on human health. The SNI is registered as a 501(c)(6) and was founded by the United Soybean Board. In addition to the industry member scientists, the SNI utilizes the services of four academic scientific advisors to provide guidance and expertise for our research efforts.

Upon learning that the CERHR intended to convene an expert panel to again evaluate soy formula the SNI commissioned the services of a third-party organization, with expertise in toxicological research, to assess the literature likely to be considered by the panel. Cantox Health Sciences International, an internationally recognized scientific and regulatory consulting firm, provided the SNI with their review and evaluation of this research. Based primarily upon their review, the SNI maintains there is no basis for the panel to diverge from the conclusion reached in 2006. For your information, comments by Cantox on the summary (pages 664 – 679, section 3.6) of the *Draft CERHR Expert Panel Report on Soy Formula* are attached.

The SNI appreciates the exhaustive and comprehensive evaluation of the literature conducted by the CERHR expert panel. The SNI recognizes that some adverse effects are reported in rodents in response to genistein, the primary isoflavone in soybeans. However, the exposure levels at which these effects are observed far exceed those to which infants are exposed via the consumption of soy infant formula. Furthermore, the effects observed in rodents are inconsistent across experimental models, of uncertain

relevance to infants, and occur primarily in response to isolated genistein in aglycone form. In contrast, infants are exposed to the glycoside form of genistein, genistin, and via the consumption of soy infant formula. Finally, in addition to the general limitations of animal studies extrapolating the results of rodents to humans is especially problematic in the evaluation of soy infant formula because of the marked differences in the metabolism of isoflavones between these two species.

Although obvious, the SNI believes it important to acknowledge that soy infant formula has produced normal short term growth and development in millions of infants since it became widely commercially available in the 1970s. This statement is consistent with the recent clinical report by the American Academy of Pediatrics (Pediatrics 2008;121:1062-8). However, the SNI also acknowledges that there is a limited amount of human research that allows assessment of the effects of soy infant formula on endpoints that go beyond those traditionally used in the assessment of infant growth and development that may be considered germane by the expert panel.

In fact, out of 74 human studies investigating the effects of soy isoflavones cited by the CERHR report, 33 were deemed of no utility and 40 of limited utility to the expert panel. Only the study by Strom et al. (reference 30 in the CERHR report) published in 2001 was deemed to be of high utility. In this retrospective cohort study of adults aged 20 to 34 years who had participated in controlled feeding studies as infants (248 were fed soy infant formula and 563 were fed cow milk formula), no statistically significant differences were observed between groups for females or males for 30 different outcome measures. Consequently, Strom et al. concluded that "Exposure to soy formula does not appear to lead to different general health or reproductive outcomes than exposure to cow milk formula."

Another example of research that did measure endpoints particularly germane to evaluation of soy infant formula was recently published (*The Beginnings Study*) by Gilchrist and colleagues from the Arkansas Children's Nutrition Center, Departments of Pediatrics and Radiology, University of Arkansas for Medical Sciences and Children's Hospital Boston, Harvard Medical School (Journal of Pediatrics, a head of print, October 19). This group ultrasonographically measured breast buds, uterus, ovaries, prostate, and testicular volumes in 40 breast fed, 41 milk formula fed and 39 soy formula fed infants at age 4 months. In brief, their results showed there were no significant feeding group effects in anthropometric or body composition. Further, all measurements in soy formula fed infants were similar to either breast fed or milk formula fed infants. Consequently, Gilchrist et al. concluded there was no evidence that feeding soy formula exerts any estrogenic effects on the reproductive organs studied.

In conclusion, it is the opinion of the SNI that the evidence in the current NTP-NIEHS report is insufficient to conclude that infants fed soy-based infant formulas are at any greater risk of developmental toxicity in comparison to infants fed breast milk or milk-based formulas. The SNI looks forward to the publication of future results from *The Beginnings Study* as these infants continue to be followed and welcomes future human research evaluating soy infant formula, as only human research has the potential to provide a meaningful assessment of health effects. The SNI firmly believes that this research will continue to show soy infant formula as a healthful,

useful and important option for mothers and pediatricians who for a variety of reasons feel that soy infant formula is the best for their infants and patients.

Sincerely

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CANTOX
HEALTH SCIENCES INTERNATIONAL

Critical Evaluation of the Preclinical Developmental Toxicity Data Included in CERHR's Expert Panel Report on Soy Formula

- Draft for Discussion -

Prepared for: Soy Nutrition Institute (SNI) supported, in part, by the United Soybean Board (USB)

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Critical Evaluation of the Preclinical Developmental Toxicity Data Included in CERHR's Expert Panel Report on Soy Formula

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Critical Evaluation of the Preclinical Developmental Toxicity Data Included in CERHR's Expert Panel Report on Soy Formula

1.0 INTRODUCTION

On March 15-17, 2006, the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) assembled an Expert Panel to conduct evaluations of the potential developmental and reproductive toxicities of soy formula and its principal isoflavone component genistein. In November 2006, after a period for public comment, CERHR released draft NTP Briefs on Genistein and Soy Formula that presented the NTP's interpretation of the potential for genistein and soy formula to cause adverse reproductive and/or developmental effects in exposed humans. While the Genistein and Soy Formula Expert Panel's findings were not finalized, the Panel did state the following for genistein and soy formula:

Genistein – Expert Panel Conclusions

"Even though there is a paucity of available human data on exposure to purified genistein, the Expert Panel expresses negligible concern for reproductive and developmental effects from exposure of adults in the general population. The most highly exposed human population reported is Japanese adults with ingestion of total genistein (free and complexed) of approximately 0.43 mg/kg body weight (bw)/day. However, adverse effects in rodent studies were not observed at levels below 35-44 mg/kg bw/day. Therefore, the Expert Panel feels that under current exposure conditions, adults would be unlikely to consume sufficient daily levels of genistein to cause adverse reproductive and/or developmental effects."

"The Expert Panel expresses negligible concern for adverse effects in neonates and infants who may consume up to 0.01-0.08 mg/kg bw/day of genistein aglycone contained in soy formula. One member of the panel did not agree with this conclusion and felt that a higher level of concern was warranted. It is noteworthy that about 1% of total genistein in soy formula is present in its uncomplexed form, i.e., the aglycone."

Soy Formula – Expert Panel Conclusion

"There are insufficient human or experimental animal data available to permit a determination of the developmental or reproductive toxicity of soy infant formula."

CERHR did not complete its evaluation, finalize the briefs, or issue NTP-CERHR monographs on isoflavones. Since 2006, a significant number of new publications examining human exposure or potential reproductive and/or developmental toxicity associated with isoflavones

have been published. As such, CERHR determined that updated evaluations of genistein and soy formula were necessary. However, their current evaluation concentrates only on soy formula and the potential developmental toxicity of its predominant isoflavone constituents including, genistein, daidzein, and glycitein. Furthermore, the updated evaluation does not take into account the potential reproductive toxicity of genistein following exposures during adulthood as was included in the 2006 evaluation. CERHR omitted this risk characterization from the current evaluation because the assessment of reproductive effects of genistein following exposures to adults was not considered relevant to the consideration of soy formula use in infants during the 2006 evaluation.

The updated Expert Panel report entitled *"Draft CERHR Expert Panel Report on Soy Formula"* encompasses the undertaking of a 14-member panel comprised of government and non-government scientists, aided by NTP staff. According to this report, the CERHR Expert Panel intends to utilize the *"Draft CERHR Expert Panel Report on Soy Formula"* to reach conclusions on (1) the strength of scientific evidence that soy formula or its isoflavone constituents are developmental toxicants based on data from *in vitro*, animal, or human studies; (2) the extent of exposures in infants fed soy formula; (3) the assessment of the scientific evidence that adverse developmental health effects may be associated with such exposures; and (4) knowledge gaps that will help establish research and testing priorities to reduce uncertainties and increase confidence in future evaluations. The Expert Panel is expected to finalize their report and reach these conclusions for soy formula at a public meeting on December 16-18, 2009.

Given the pending release of the final Expert Panel report on the potential human developmental effects of soy formula, the Soy Nutrition Institute (SNI) requested that Cantox conduct a review of the *"Draft CERHR Expert Panel Report on Soy Formula"*. Specifically, Cantox agreed to examine Section 3.6, Summary of Developmental Toxicity Data, and provide a detailed response to the Panel's assessment of preclinical data utility contained therein. In addition, Cantox reviewed Section 3.3 (Experimental Animal Studies on the Individual Isoflavones Found in Soy Formula) and Section 3.4 (Experimental Animal Studies of Soy Formula or Other Soy Exposures during Development), in parallel with Tables 159, 160, and 162 for consistency between written and tabulated summaries. With the exception of the study conducted by Jefferson *et al*. (2005), studies reviewed by Cantox were limited to those published since 2006 and considered by the Expert Panel to be of "high" utility.

The following report provides a summary of our assessment of the utility and consistency of the preclinical developmental toxicity data as described by the Expert Panel in Sections 3.3, 3.4, and 3.6 and Tables 159, 160 and 162.

2.0 EVALUATION OF PRECLINICAL DEVELOPMENTAL TOXICITY DATA UTILITY AND CONSISTENCY

2.1 Considerations

As indicated in Section 1.0, with the exception of the study conducted by Jefferson *et al*. (2005), studies reviewed by Cantox were limited to those published since 2006 and considered by the Expert Panel to be of "high" utility. In addition, Cantox took into account the following excerpts from Section 3.6.2 and Section 3.6.3 of the draft Expert Panel report when reviewing Sections 3.3 and 3.4 in parallel with Tables 159, 160, and 162 for consistency between written and tabulated data summaries:

With respect to experimental animal studies on the individual isoflavones found in soy formula:

"Studies reporting the most sensitive and apparently treatment-related developmental effects are summarized in Table 159 and Table 160 for oral and parenteral exposures in mice, for oral and parenteral exposures in rats... In these tables, dose levels have been converted to mg/kg bw. In general, the most complete information was available from parenteral exposure studies in mice and oral exposure studies in rats. In cases where doses were converted to mg/kg bw/day values, ranges were often estimated over periods of gestation or lactation or in different stages of the offspring's life. In order to simplify dose comparisons, exposure ranges were averaged in summaries of developmental toxicity effects."

With respect to experimental animal studies of soy formula or other soy exposures during development:

"Experimental animal studies are summarized in Table 162, 7 of the studies were judged to have high utility and more than 25 additional studies were found to be of limited utility for the evaluation process."

2.2 Data Evaluation

Jefferson *et al*., 2009 [243][1] (oral, mice)

In comparing the written summary on pages 363-365 with the entry for this study in Table 159 (page 688), the following inconsistencies were noted:

[1] The number in brackets represents the reference number as cited in the reference list of the draft expert panel report.

1. 2nd row of table entry for this study - The NOEL (12.5 mg/kg bw/day) and LOEL (≥25 mg/kg bw/day) for ↑ number of abnormal estrous cycles are incorrect; the NOEL should be 6.25 mg/kg bw/day, while the LOEL should be 12.5 mg/kg bw/day).

2. 4th row of table entry for this study - The LOEL for ↑ uterine weight of 25 mg/kg bw/day is incorrect; the LOEL for this endpoint should be 75 mg/kg bw/day.

In addition, we noted the following with respect to the table entry

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1. It is unclear for table entries with more than one endpoint listed which endpoint the specified NOEL and LOEL applies to. For example, in the 2nd row, ↑ uterine weight and ↑ number of abnormal estrous cycles are listed together and only one NOEL and LOEL are provided. Only after we referred to the study summary in Section 3.3.1.1.4 was it clear that the NOEL of 12.5 mg/kg bw/day and the LOEL of ≥25 mg/kg bw/day applied only to the uterine weight endpoint.

Jefferson *et al*., 2009 [533] (parenteral, mice)

In comparing the written summary on pages 376-378 with the entry for this study in Table 159 (page 690), the following inconsistencies were noted:

1. 1st row of table entry for this study – the ↓ number of embryos collected per mouse following hCG administration endpoint should have time points specified as well. On page 378 the study summary states: "The mean numbers of embryos per mouse collected at 24 and 48 hours after hCG administration were not different between groups; however, after 72 and 92 hours after hCG administration, there was a significant reduction in the number of embryos retrieved…"

2. The 1st row table entry, in the Endpoints column includes the entry "↓ number and size of implantation sites in mice who were recipients of transferred blastocysts obtained from untreated mice"; however, the text summary does not specify the number of implantation sites observed in either the control or genistein-treated groups. Instead, only a vague descriptor ("few") is used in reference to the number of implantation sites in the genistein-treated group, while no mention is made of the number of implantation sites in the control group.

NCTR, 2008 [576] (oral, rats)

In comparing the written summary on pages 465-474 with the entry for this study in Table 160 (page 697-698), the following inconsistency was noted:

1. Ovarian follicle counts were also unaffected by genistein treatment, thus, this endpoint should be included in the last row of the table entry (No effect).

McClain *et al*., 2007 [551] (oral, rats)

In comparing the written summary on pages 405-409 with the entry for this study in Table 160 (page 699), the following inconsistencies were noted:

1. Page 406, line 26 of the text summary – should read "1000 mg/kg bw/day" instead of "100 mg/kg bw/day".

2. 2nd row of table entry for this study reads as follows:

Endpoints	NOEL	LOEL
visceral malformations (artery origin variant)	150	1000 (on litter incidence basis)

Our review of the original McClain *et al*. (2007) article for further clarification on this endpoint led to questions regarding interpretation of the findings of this study in the CERHR report, particularly those related to the pup visceral examination. Table 5 of the McClain *et al*. article, a portion of which is shown below, summarizes the results of pup visceral examinations.

	Dose group (mg/kg/day)			
	0	20	150	1000
Pup weights (g)				
Day 1	5.6	5.1	5.9	5.2
Day 4	7.9	5.7	7.8	7.4
Day 6	10.1	7.4	10.1	9.5
Visceral examinations (pups)	71	68	91	72
Total abnormalities				
Pup incidence (%)	1 (1.4)	0 (0)	1 (1.1)	2 (2.8)
Litter incidence (%)	1 (12.5)	0 (0)	1 (11.1)	1 (14.3)
Total variations				
Pup incidence (%)	9 (12.7)	5 (7.4)	12 (13.2)	12 (16.7)
Litter incidence (%)	5 (62.5)	4 (50)	6 (66.7)	5 (71.4)
Total retardations				
Pup incidence (%)	0 (0)	0 (0)	2 (2.2)	5 (6.9)
Litter incidence (%)	0 (0)	0 (0)	1 (11.1)	1 (14.3)
Skeletal examinations (pups)	70			73

Additional findings, described solely in the journal text (section 3.2.5), are summarized in Tables 2-1 and 2-2.

Table 2-1. Findings expressed as *percent* of pups affected

Described as	Dose group (mg/kg/day)			
	0	**20**	**150**	**1000**
Blood vessel variation: "artery origin variant"	0%	1.5%	5.5%	5.6%*

*Found by McClain *et al*. (2007) to be significant at this level with respect to litter incidence (more than half of the litters affected at 1000 mg/kg), but not at 150 mg/kg/day or below.

Table 2-2. Findings expressed as number of pups affected

Described as	Dose group (mg/kg/day)			
	0	**20**	**150**	**1000**
Retardation finding: "thymus remnant"	0	0	2 (from 1 litter)	5 (from 1 litter)
Thymus hypoplasia	0**	1	0**	0**
Other variations: "innominate artery missing, innominate artery shortened"	No clear dose-dependency and considered not related to treatment			
Other isolated findings considered not related to treatment: Missing testicle and epididymis	0**	0**	1	0**
Displaced kidney	0**	1 pup (dose level unclear)		
Testicle, small	0**	0**	0**	2 (from 1 litter)
Convoluted ureter and persistent ductus botalli	1	0**	0**	0**
Red dots on liver or kidney (considered incidental)	0**	0**	all or most pups from 2 litters	0**

*presumed, not specified.

Interestingly, although these visceral variations are described in detail in the CERHR summary for this study (Section 3.2.5, page 406), only the blood vessel variation "artery origin variant" was included in Table 160 (page 699). We found this to be somewhat misleading because it suggests that, by virtue of having reached statistical significance with respect to litter incidence (*i.e.*, occurring in more than half of the litters from the 1000 mg/kg bw/day group, per McClain *et al.*), the finding of "artery origin variant" is related to administration of the test substance and/or of significance to human health. Table 160 fails to qualify that the significance of this finding is unclear, especially considering that:

a. Slight maternal toxicity was noted at this dose level (↓body weight and ↓food consumption at 1000 mg/kg bw/day);

b. The litter incidence of total visceral ***abnormalities, variations, and retardations*** was generally comparable across all groups;

c. The pup incidence of total (visceral) ***abnormalities*** was comparable across all groups, occurring in 2 pups (2.8%) from the 1000 mg/kg bw/day group *vs*. 1 pup (1.4%) in the control group;

d. The pup incidence of total (visceral) ***variations*** was only marginally higher in the 1000 mg/kg bw/group, occurring in 12 pups (16.7%) *vs.* 9 pups (12.7%) in the control group; and

e. The only visceral finding that was observed in the treated but not the control group was the pup incidence of total (visceral) ***retardations***, occurring in 2 pups (2.2%) from the 150 mg/kg bw/day group and 5 pups (6.9%) from the 1000 mg/kg bw/day group.

It is worth noting that the authors did not describe the types of variations observed in the control group pups.

Latendresse *et al*., 2009 [412] (oral, rats)

In comparing the written summary on pages 572-573 with the entry for this study in Table 160 (page 711), the following inconsistency was noted:

1. Last row of table entry for this study, beginning No effect: – In addition to those endpoints listed, it should also be noted that there were no effects on the incidence of adenoma, adenocarcinoma, fibroma, or fibroadenoma in F1T140 males.

Liu *et al*., 2008 [631] (oral, rats)

In comparing the written summary on pages 447-451 with the entry for this study in Table 162 (page 719), the following inconsistencies were noted:

1. Animal model and study design column – the control dose is not represented.

2. In the Endpoints column, last line under Diets containing soy isoflavones should read: ↓ ER mRNA expression in **uterus** (≥150 mg/kg bw/day), rather than ↓ ER mRNA expression in **ovary** (≥150 mg/kg bw/day)

Mardon *et al.*, 2008 [668] (oral, rats)

In comparing the written summary on pages 643-645 with the entry for this study in Table 162 (page 720), the following inconsistencies were noted:

1. Typographical errors:

    a. The word “not” is missing from the third line under Endpoints for this study in Table 162 (should read “...but <u>not</u> at 24 months...”).

2. None of the effects observed in the F_0 generation (significantly lower uterine weights than control; much higher DPD urinary excretion when compared to F_1 generation at 3 and 6 months) appear in Table 162.

Fujioka *et al*., 2007 [676] (oral, mice)

In comparing the written summary on pages 652-654 with the entry for this study in Table 162 (page 724), the following inconsistencies were noted:

1. The statement that appears under Endpoints for 0.08% genistein in Table 162 (page 724) "↓ lean body mass/fat mass in females fed genistein" does not match the results that appear in Table 157 on page 653. Table 157, shown below, indicates that fat mass in females receiving 0.08% genistein was lower than control, but the results of lean body mass are not reported, presumed (by the authors) to be of no significant difference from control.

Table 157. Effects of Daidzein or Genistein on Organ Weights and Bone Formation in Immature Mice (Fujioka et al., 2007)

Parameter	0.08% Daidzein		0.08% Genistein	
	Male	Female	Male	Female
Terminal Body Weight	↓	↔	↓	↔
Lean Body Mass / Fat Mass	↔/ ↔	↔ / ↔	↔ / ↔	- / ↓
Food Intake	↔	↔	↔	↔
Spleen, Kidney, Testis or Uterus Weight	↔	↔	↔	↔
Thymus Weight	↓	↓	↓	↓
Liver Weight	↔	↔	↔	↓
Whole Body BMD / BMC	↑ / ↑	↑ / ↑	↔/ ↔	↔/ ↔
Lumbar Spine BMD / BMC	↑ / ↑	↔/ ↔	↔/ ↔	↔/ ↔
Whole Femur BMD / BMC	↑ / -	↓ / ↓	↑ / -	↓ / -
Proximal Femur BMD / BMC	↔/ -	↓ / -	↔/ -	↓ / -
Middle Femur BMD / BMC	↑ / -	↔/ -	↑ / -	↔/ -
Distal Femur BMD / BMC	↑ / ↑	↓ / ↓	↑ / -	↔/ -
Periosteal MAR	↔	↔	↔	↔
MS/MB	↑	↔	↑	↔
BFR/BS	↑	↓	↑	↔
Plasma genistein	↔	↔	↑	↑
Plasma daidzein & equol	↑	↑	↔	↔
Plasma testosterone or 17*β*-estradiol	↔	↔	↔	↔

↔No significant difference from control value
↑ Greater than control value
↓ Less than control value
- Not reported, presumed to be no significant difference from control value
From Fujioka et al., 2007 [*676*]

2. The statement that appears under Endpoints for 0.08% daidzein in Table 162 "alterations in a variety of bone formation measures in males and/or females" is vague and fails to acknowledge that most parameters related to bone formation were unchanged or increased when compared to control values.

3. The word "daidzein" is missing from the next to last line under Endpoints (No effect); it should read "...liver weight in females fed **daidzein**; plasma..."

Ruhlen *et al*., 2008 [645] (oral, mice)

In comparing the written summary on pages 605-607 with the entry for this study in Table 162 (pages 724-725), the following inconsistencies were noted:

1. In the summary (pages 605-607), endpoints are listed mostly as they relate to animals receiving the PMI 5K96 soy-free diet compared to the soy-based diet (*e.g*., higher serum estradiol); Table 162 (page 724) lists them as they relate to animals receiving the PMI 5008/5001 soy-based diet compared to the soy-free diet (*e.g*., lower serum estradiol).

2. Line 13 of page 605 should say "the pups...remained on this feed **after** weaning" rather than "the pups...remained on this feed **until** weaning".

3. Page 724, Endpoints column, beginning F1 offspring of dams fed soy-based diet (PMI 5008 or PMI 5001) compared to soy-free diet (PMI 5K96) – specifies ↓ PND 90 body weight (but no effect at PND 20 or PND 26). The summary presents it as "PMI 5K96-fed males and females were significantly heavier..." on postnatal day (PND) 90 but not on PND 20 (weaning) or PND 26. However, the summary also states that "on PND 26, females fed PMI 5K96 were significantly heavier..." (lines 37-38, page 606)

4. Page 724, Endpoints column, beginning F1 offspring of dams fed soy-based diet (PMI 5008 or PMI 5001) compared to soy-free diet (PMI 5K96) – Later onset of fertility in females, which although listed as a significant effect, was slight (46.6 days *vs*. 44.7 days in control females).

5. The written summary does not specifically indicate that uterine <u>weight</u> was lower at PND 26 in females receiving the soy based diet. This effect is described as "on PND 26, females fed PMI 5K96 ...had significantly larger uteri..." (lines 37-38, page 606). It has not been determined at this time whether the original journal article uses weight and size interchangeably. However, it should be noted that it is possible for the size of an organ to change without altering its absolute weight, and *vice versa*.

6. The summary (page 606, lines 27-28) states that, on PND 90, "serum leptin was 121% higher in males and 174% higher in females fed PMI 5K96 compared to males and

females fed PMI 5008/5001." It is unclear from the summary whether these differences were statistically significant and should be included in Table 162.

Jefferson *et al*., 2005 [531] (parenteral, mice)

In comparing the written summary on pages 371-375 with the entry for this study in Table 159 (page 690), the following inconsistencies were noted:

1. Endpoints unaffected by genistein (serum progesterone and estradiol, number of plug-positive mice, number of ovulated oocytes following hCG administration at 4 months, corpora lutea at 6 weeks) are not listed in Table 159 (page 690).

2. There is a lack of clarity in how the findings of this study are represented in Table 159. Specifically:

    a. "Distribution of females in various stages of estrous cycle at 2 months of age" is too vague a statement, especially since there is no indication that the differences among groups were statistically significant. As Table 101 (page 373 and below) illustrates, at 2 months of age, diestrus was extended in 0/8 animals in each the control group and the high-dose (50 mg/kg bw/day genistein) groups, and in a few animals in the low- and mid-dose groups (2/8 and 4/8, respectively). Extended estrus was observed in 0/8 control animals, and 1/8, 3/8, and 6/8 animals from the low-, mid-, and high-dose groups, respectively. At 2 months, persistent estrus was noted only in 1/8 high-dose group animals. At 6 months, the incidence of extended diestrus was highest in the control and low-dose groups (5/8 in each), and the incidence of extended estrus was only marginally higher in genistein-treated animals (not more than 2/8 animals). Persistent estrus, not observed in the control or low-dose groups and in only 1/8 animals from the mid-dose group, was considerably higher in the high-dose group, occurring in 5/8 animals.

Table 101. Estrous Cyclicity Effects in Mice Treated as Neonates with Genistein (Jefferson et al., 2005)

	Genistein, mg/kg bw/day					
Endpoint	**0**	**0.5**	**5**	**50**	**BMD_{10}[a]**	**$BMDL_{10}$**
Evaluated at 2 months of age						
Extended diestrus	0	2	4	0	2	1
Extended estrus	0	1	3	6	9	6
Persistent estrus	0	0	0	1	49	28
Evaluated at 6 months of age						
Extended diestrus	5	5	4	1	Not calculated	
Extended estrus	0	1	2	2	33	14
Persistent estrus	0	0	1	5	17	10

Data shown as number of mice with the indicated effect of a total of 8/group.
[The authors noted "* Endpoints that demonstrate a statistically significant difference among the dose categories using Fisher's Exact Test ($P < 0.05$)."]
[a]See the footnote to Table 100 for an explanation of the use of benchmark dose in this report. A probit model was used. The 50 mg/kg bw/day dose was omitted for benchmark dose modeling of extended diestrus at 2 months of age.
From Jefferson et al.,2005 [*531*].

b. Genistein-treated animals are reported to have had "↓ pregnancies (number of dams delivering live pups)" and "↓ number of live pups born to dams" at 2, 4, and 6 months of age. However, Table 102 of the written summary (page 374) shows no statistically significant differences between the control group and the 0.5 mg/kg bw/day genistein group in either of these parameters (No. pregnant/plug positive animals; live pups/dam) at any time point. In addition, Table 159 does not explain that, in animals receiving 50 mg/kg bw/day, these parameters were not measured at 4 or 6 months because, at 2 months, none of the dams in this group gave birth to live pups, and a second group treated with 50 mg/kg bw/day on PND 1-5 also failed to deliver live pups. Statistically significant differences in these parameters were observed only in the 5 mg/kg bw/day dose group. In this group, the No. pregnant/plug positive animals was lower at 2 months. Although the 5 mg/kg bw/day group had fewer live pups/dam, the differences were not significant when each time period was analyzed separately, only when the 3 time points were combined.

c. "↓ corpora lutea per dam at 4 months of age" is not an accurate statement. According to the written summary and Table 102 therein, corpora lutea were measured only at 4 months. No corpora lutea were found in the 50 mg/kg bw/day group. A greater number of corpora lutea were found in the 0.5 and 5 mg/kg bw/day groups compared to control; at 5 mg/kg bw/day the number was approximately twice that of the control (~18 *vs*. 9, respectively). It is unclear from Table 102 which of these differences was statistically significant; the written summary states that "...mice in the 5 mg/kg bw/day group had significantly more corpora lutea, but none were observed in mice of the 50 mg/kg bw/day group".

3. An additional study is described in the written summary but was not included in Table 159. This study was conducted to further assess implantation defects and pregnancy loss in mice treated with 50 mg/kg bw/day genistein.

4. In its evaluation of the utility (adequacy) of this study, CERHR has specified that this study "...shows that a relatively low genistein dose of 0.5 mg/kg bw/day has deleterious consequences." However, aside from (1) earlier vaginal opening (with no significant effect on mean day of vaginal opening); (2) slightly fewer (not reported to be statistically significant) live pups/dam at 2 months and 6 months, but not at 4 months; and (3) more corpora lutea at 4 months, 0.5 mg/kg bw/day of genistein did not appear to have any effect or consequences on any of the measured parameters.

3.0 CONCLUDING REMARKS

Based on this review, it is the opinion of Cantox that:

1. There is a lack of clarity and/or completeness in how the findings of a number of studies are represented in Tables 159, 160, and 162. For example, statements regarding endpoints with multiple parameters were found to be too vague. While alterations in parameters were noted, the tables failed to acknowledge other related parameters that were unchanged compared to the control group (McClain *et al*., 2007; Fujioka *et al*., 2007; Jefferson *et al*., 2005). In addition, for table entries with more than one endpoint listed it is unclear which endpoint the specified NOEL and LOEL applies to (Jefferson *et al*., 2009).

2. The listing of endpoints under "No effect" was incomplete for several studies (NCTR, 2008; Latendresse *et al*., 2009; Jefferson *et al*., 2005).

3. CERHR appears to have been rather selective in their interpretation of study findings and determination of study utility. For example, our review of the original McClain *et al*. (2007) article led to questions regarding interpretation of the findings of this study in the CERHR report, particularly those related to the pup visceral examination. Although a number of visceral variations (*e.g*., thymus remnant, innominate artery missing, innominate artery shortened) are described in detail in the CERHR summary for this study (Section 3.2.5, page 406), only the blood vessel variation "artery origin variant" was included in Table 160 (page 699). We found this to be somewhat misleading because it suggests that, by virtue of having reached statistical significance with respect to litter incidence (*i.e.*, occurring in more than half of the litters from the 1000 mg/kg bw/day group, per McClain *et al.*), the finding of "artery origin variant" is related to administration of the test substance and/or of significance to human health. Table 160 fails to qualify that the significance of this finding is unclear.

Furthermore, in its evaluation of the utility (adequacy) of the Jefferson *et al*. (2005) parenteral study, CERHR has specified that this study "...shows that a relatively low genistein dose of 0.5 mg/kg bw/day has deleterious consequences." However, aside from (1) earlier vaginal opening (with no significant effect on mean day of vaginal opening); (2) slightly fewer (not reported to be statistically significant) live pups/dam at 2 months and 6 months, but not at 4 months; and (3) more corpora lutea at 4 months, 0.5 mg/kg bw/day of genistein did not appear to have any effect or consequences on any of the measured parameters.

4. There appears to be little reason for the CERHR to reach conclusions that vary greatly from those previously reported (refer to Section I.0) based upon the results of preclinical developmental toxicity data published since CERHR's evaluation in 2006